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Prevalence and risk factors for canine leishmaniasis in Mashhad, North-East of Iran

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Abstract

The present study was carried out to determine the seroprevalence and risk factors of canine leishmaniasis in a population of owned and strayed dogs in Mashhad, North-east of Iran. Of 300 serum samples obtained from dogs, 8.67% (26/300) were positive for *Leishmania infantum* by Indirect Florcense Antibody Test (IFAT). The sero-prevalence rates in owned and stray dogs were 9.5% (19/200) and 7% (7/100), respectively. Significant differences were observed between the age groups ($p<0.05$); dogs less than one year old showed the highest seroprevalence rate (17.4 %). The seroprevalence rate for leishmania infection in male and female dogs were 10.43 % (17/163) and 6.57% (9/137), respectively. In addition, the seroprevalence rate for leishmania infection in small and large breeds of dogs were 10.5% (6/57) and 8.2% (29/243), respectively. No significant difference was found between sex and breed of the studied dogs. The number of lymphocytes were significantly higher in the leishmania-infected dogs than dogs which were not infected ($p<0.05$). Packed cell volume (PCV) determination revealed a high frequency of non-regenerative anaemia in leishmania infected dogs (52.6%). According to the results of the present study, the seroprevalence rate of canine leishmaniasis in the owned and stray dogs from Mashhad, North east of Iran, is relatively high. In addition, non regenerative anemia is the most frequent hematological finding in the leishmania-infected dogs, which must be taken into account when dealing with diagnosis of canine leishmaniasis in endemic regions.

Keywords: canine leishmaniasis, hematology, Mashhad, Iran, seroprivalence

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Introduction

Visceral leishmaniasis, caused by *Leishmania infantum*, is a zoonotic disease found in Latin America, Europe, Asia and Africa (Quinnel *et al*., 2001).The transmission cycle of *L. infantum* is thought to be zoonotic with canids acting as reservoir hosts. It is widely believed that human disease in leishmaniasis-endemic areas is associated with the presence of domestic dogs *(Canisfamiliaris)* and the parasite is transferenceby phlebotomine sandflies (Diptera Psychodidae) (Mazloumi Gavgani *et al*., 2002 andda Costa-Val *et al*., 2007). From the epidemiological point of view, the canine visceral leishmaniasis is considered to be more important than the human disease, due to its higher prevalence and the fact that infected asymptomatic and symptomatic dogs may be a source of infection for sandflies even after successful clinical therapy (Cavaliero *et al*., 1999; Reis *et al*., 2006 andMoshfe *et al*., 2008). Therefore, one of the approaches to reduce the incidence of human visceral leishmaniasis (also known as kala-azar) is to cull infected dogs (Reithinger *et al*., 2002).The percentage of infected dogs living in an area where canine visceral leishmaniasis is endemic has major public health implications. Therefore, the estimationof prevalence and incidence of canine infection by a reliable method is an important epidemiological parameter (Quinnel *et al*., 2001). Since the sensitivity and specification for IFAT are approximately 100% (Mancianti *et al*., 1995; Mettler, *et al*., 2005), the test is considered by World Organization for Animal Health (OIE) as a serologic reference method (Gradoni and Gramiccia, 2000). Infected dogs may present wide range of clinical profiles, from apparently healthy to critically ill by manifestations including fever, anemia, progressive loss of weight, pale mucous membranes, epistaxis, lymphadenomegaly and hepatosplenomegaly (Koutinas *et al*., 2001, Ikedagarcia *et al*., 2008). Dermatological alterations are very frequent in dogs with visceral leishmaniasis and they may occur in the absence of other symptoms (Ikedagarcia *et al*., 2008). Clinical evolution of visceral leishmaniasis in naturally infected dogs, also promotes clear alterations in hematological parameters (Reis *et al*., 2006). Some authors report that moderate, non-regenerative anemia is a frequent clinical sign in canine visceral leishmaniasis (Abranches *et al*., 1991a; Ciaramella *et al*., 1997; Koutinas *et al*., 1999; Reis *et al*., 2006). In contrast, others (Cabassu *et al*., 1988; Denerolle *et al*., 1996 and Amusategui *et al*., 2003) observed a relatively low percentage of dogs with anemia. In addition, a great variability in the leukogram of dogs with leishmania infection has been reported (Anosa and Idowu, 1983; Moreno *et al*., 1998; Koutinas *et al*., 1999; Juttner *et al*., 2001 andAmusategui *et al*., 2003). Hyperproteinemia, hypoalbuminemia, hyperglobulinemia and altered albumin : globulin ratio are the major changes in serum biochemical parameters of Canine leishmaniasis (Pasa *et al*., 2005).

Canine leishmaniasis is an endemic disease in Iran (Mohebali, *et al*., 2005), and its seroprevalence has been reported in various parts of Iran (Hamidi, *et al*., 1982; Mazloumi Gavgani, *et al*., 2002; Mohebali, *et al*., 2005 andMoshfe, *et al*., 2008). To the best of our knowledge, this is the first study on Canine leishmaniasis carried out in Mashhad (Northeast of Iran) and our objectives were to: (1) determine the prevalence of canine leishmaniasis in our area and (2) to determine the risk factors associated with the likelihood of canine leishmaniasis.

Materials and methods

Study area

The study was conducted in Mashhad, one of the largest cities located in Northeast of Iran. The city is located at 36.20º north latitude and 59.35º east longitude, in the valley of the Kashaf River, between the two mountain ranges of Binalood and Hezar-masjed. Mashhad is situated at an altitude of

985m above sea level and its climate is semi-arid with cold winters and moderate summers and average annual rainfall of 241.3 mm. The city's mean annual temperature ranges from 8 to 20°C (http:// amar.org.ir)

Dog's demographic information

The investigation was carried out on 200 owned dogs and 100 stray dogs. The dogs' breed[1] (small [<20 lb]; or large [≥20 lb]), sex (male or female) and age (less than 1 year old, 1-2 years old, 2-5 years old and greater than 5 years old) were recorded to determine whether they were associated with the likelihood of canine leishmaniasis.

Haematological analysis

Blood samples for haematological and serological analysis were obtained by cephalic venepuncture with 2 mL blood being collected into a vial containing ethylene diamine tetraacetic acid (EDTA). A further 3 mL aliquot was collected into tubes without anticoagulant, centrifuged at 800 g for 10 min, and the separated sera were stored at -20°C. Anti coagulated blood obtained from owned dogs was analyzed shortly after collection for hematological analysis. PCV was determined by micro-haematocrit method (Jain, 1993). Morphological characteristics of the blood cells and differential leukocyte counts were obtained by blood smear analysis after prior staining by routine methods. Stained blood smear was also used for estimation of platelet numbers. Platelet numbers were reported as decreased (≤9 platelets/ 100 X oil immersion field of view), within the reference interval (10-25) or increased (≥26) (Stockham and Scott, 2002). Determination of total protein was performed by means of refractometry.

Serological analysis

In order to determine prevalence of *L. infantum* infection, we used indirect immunofluorescent assay (IFAT). The antibodies to *L. infantum* were detected by IFAT using MegaScreen® FLUOLEISH (MegacorDiagnostik GmbH, Lochauer Str. 2 A-6912, Hoerbranz, Austria). A titer of at least 1:50 was considered positive according to MegaScreen's instructions.

Statistical analysis

Statistical analysis was conducted using SPSS for windows (release 16, SPSS Inc, Chicago, Ill) with a *p* value of <0.05 as statistically significant.

Chi-squared tests (x^2) were conducted to examine whether the dogs' breed, sex, age and life style, (stray or owned) were associated with canine leishmaniasis infection. Independent-samples *T* test was used to compare hematology parameters between infected dogs which were not infected.

Results

Dog's demographic information

With regard to the qualitative variables, our population included dogs of 17 breeds (mixed, German Shepherd, Boxer,Rottweiler, Terrier, Spitz, Dalmatian, Dachshund, Chihuahua, Pekingese, Bulldog, Great Dane, Doberman,Welsh corgi, Gale, Spaniel and Sheepdog), both sexes (163 males and 137 females) and different ages (46, Less than 1 year old; 53, 1-2 years old; 143, 2-5 years old and 58, greater than 5 years old).

Serological evaluation

The sero-prevalence rate in titers1: 50and above was 8.67% (26/300). Of the 200 serum sampled collected from owned dogs, 9.5% (19/200) were positive by IFAT and in stray dogs, 7% (7/100) were positive for sero-prevelence.No statistically significant difference was seen between these two groups of dogs (X^2= 0.526; *p*=0.468).11 (42.3%) of the sero-positive dogs showedat least one clinical sign including cutaneous lesions, lymphadnopathy, anemia, loss of weight and epistaxis, and no clinical sign and symptom wasseen in 15(57.7 %) of seropositive dogs.

The sero-prevalence values among male

1. According to the official breed standard from the American Kennel Club (www.akc.org/breeds).

and female animals were 10.43 % and 6.57%, respectively (Table 1). No statistically significant difference between canine *Leishmania* infection and gender was observed (X^2=1.401; p=0.237). Referring to animal age groups, the highest sero-prevalence (17.4%) was found in dogs less than 1 year old and the lowest values (4.9%) in dogs 2-5 years old (Table 2). A significant difference was observed betweenage groups (X^2=8.604; p= 0.035).There was a great variety of breeds of dogs in this study and we classified them into two groups: small or toy and large breed dogs. No significant difference was found between the sero-prevalences of *Leishmania infantum* in small 10.5% (6/57) and large breed 8.2% (29/243) dogs (X^2=0.307; p= 0.579).

Table 1. Sero-prevalence of canine leishmania infection by gender in Mashhad.

Gender	Number of dogs tested	No of IFAT positive (≥1:50)	Prevalence (%)
Male	163	17	10.43
Female	137	9	6.57
Total	300	26	8.67

Table 2. Sero-prevalence of canine leishmania infection by age groups in Mashhad.

Age groups (years)	Number of dogs tested	No of IFAT positive (≥1:50)	Prevalence (%)
< 1	46	8	17.4
1-2	53	7	13.2
2-5	143	7	4.9
>5	58	4	6.9
Total	300	26	8.67

Table 3. Mean ± SD of hematology parameters in infected and non infected dogs.

Parameters	infected dogs	Non infected dogs
PCV (L/L)	0.38 ± 0.094	0.41 ± 0.072
WBC (10^9/L)	12.1 ± 5.86	10.85 ± 6.24
Neutrophil (10^9/L)	8.02 ± 3.89	7.23 ± 5.17
Eosinophil (10^9/L)	0.65 ± 0.68	0.75 ± 0.97
Lymphocyte (10^9/L) *	3.2 ± 2.48	2.12 ± 1.28
Monocyte (10^9/L)	0.65 ± 0.6	0.45 ± 0.9
Plasma total protein (g/L)	74 ± 12	72 ± 9

* Significant difference between groups (p<0.05)

Hematological results in Leishmania-infected and -non infected dogs

Hematology parameters were determined in owneddogs (*n=200*).The number of lymphocytes was significantly higher in the infected dogs than those in the non infected dogs (p<0.05). The results of different hematological parameters in the infected and non-infected dogs have been shown in Table 3.

PCV determination revealed a high frequency of anaemia in Leishmania-infected dogs: 10/19 dogs (52.6%) presented with PCV (mean value 31.42%; standard error: 1.71%; reference range 37–55%) below reference values (Meinkoth and Clinkenbeard, 2000). On the basis of polychromatophil counts, the anemia was shown to be normocytic, normochromic and non-regenerative.

Platelets count estimation showed normal platelets count in 17 infected dogs (89.5%), thrombocytopenia in one dog (5.3%) and thrombocytosis in one of the dogs (5.3%).

16 dogs presented normal leukocyte counts, while two had leukocytosis and one had leukopenia.Normal plasma total protein was observed in 13 dogs (63.16%), while 5 (31.58%) dogs had hyperproteinemia and 1 dog had hypoproteinemia.

Discussion

In most endemic areas, domestic dog (*Canis familiaris*) is the main reservoir for *L. infantum* (Mazloumi Gavgani *et al*., 2002 and Mohebali *et al*., 2005). Determination of the prevalence of canine leishmaniasis by a reliable method is necessary to define controling measures for zoonotic visceral leishmaniosis (Tesh, 1995). Serological methods are highly sensitive and isnotinvasive. They are comparatively more suited for diagnosing visceral leishmaniasis in endemic regions (Alborzi *et al*., 2007).

Based on our results, sero-prevalence of canine leishmaniasis in Mashhad was determined 8.67% usingthe cut-off value of 1:50 and above. The sero-prevalence rates in owned and stray dogs were 9.5 and 7%, respectively, and no significant difference was observed between these two groups of dogs. Major investigations into canine leishmaniasis in Iran have been carried out in North-West as well as in intermediate and South-West, but no study has been done in Mashhad (North-East of Iran).In Iran, the geographical regions with various weathers have shown different sero-prevalence ratesof *L.infantum*in dogs (Mohebali, *et al*., 2005). The highest sero-prevalence of canine leishmaniasis in Iran have been observed from the cold Northwest part, where 17.4% (Moshfe *et al*., 2008), 18.2% (Mohebali *et al*., 2005) and 21.6% (Mazloumi Gavgani *et al*., 2002) rate of sero-prevalence have been reported. On the other hand, leishmania infection rate of dogs in hot South-estern part of Iran was low (4.4%) (Mohebali *et al*., 2005).The sero-prevalence rate of canine leishmaniasis in Mashhad (8.67%) is intermediate, which suggests that the climatic factors can affect the prevalence of canine visceral leishmaniasis.

Although a greater number of males than females were affected by canine leishmaniasis, no significant difference was found among leishmania- infected and non-infected dogs with regard to gender in our study. Similar results were found by Pozio *et al*.,(1981); Abranches *et al*. (1991b); Sideris *et al*.,(1996), Bokai *et al*., (1998) and Mohebali *et al*. (2005). On the other hand, some authors have reported a predominance of leishmaniasis in men than women (Brabinand Brabin, 1992). It is probably due to the more exposure of males to the sand fly bites (Amusategui *et al*., 2003).

No significant difference was seen between small and large breeds with regard to leishmania infection in our study. Although more cases of canine leishmaniasis have been reported in large dogs (Denerolle, 1996), no breed-related predisposition to leishmaniasis exists; in fact, the disease is described in all breeds (Slappendel, 1988; Abranches *et al*., 1991b; Ferrer, 1992 and Ciaramella *et al*., 1997).The German Shepherd dog is one ofthe breeds most frequently mentioned in differentstudies. The lifestyle and exposure to sandflies are the most important factors in predispositionof dogs to leishmania infection (Ferrer, 1992).

In the current study, we found canine leishmania infection mostly in dogs less than 1 year old and the lowest sero-prevalence rate was found in dogs 2-5 years old. In contrast to the results of the present study, others (Amusategui *et al*., 2003; Mohebali *et al*., 2005 and Moshfe *et al*., 2008) have observed the highest sero-prevalence ratein older dogs.The reason of this finding is not clear.

Similar to many reports, the most common clinical sign in the infected dogs studied was skin lesions (Ciaramella *et al*., 1997; Strauss-Ayali and Baneth, 2001; Amusategui *et al*., 2003 and Solano-Gallego *et al*., 2004).

Fifty-two percent (10/19) of our infected dogs were presented with non-regenerative anemia as has been observed by other authors (Abranches *et al*., 1991a; Ciaramella *et al*., 1997; Koutinas *et al*., 1999 and Reis *et al*., 2006). Some authors reported that moderate,

non-regenerative anemia is an almost constant finding in canine leishmaniasis(Keenan *et al*., 1984; Ferrer, 1992 and Binhazim *et al*., 1993), but others (Cabassu *et al*., 1988 and Denerolle 1996), observed a relatively low percentage of dogs with anemia (20−30%).The possible causes of the anemia are blood loss due to epistaxis and skin ulcerations, haemolysis, generalized inflammation, renal insufficiency and bone marrow hypoplasia oraplasia (da Costa-Val *et al*., 2007).The non-regenerative feature of the anemia can be attributed to infection of the bone marrow by *L. infantum*, inducing infiltration by lymphocytes, plasma cells and macrophages that could contribute to a decrease in erythrocyte production. The azotemia encountered in most of the dogs infected by *L. infantum*could also contribute to the anemia, as this would alter erythropoietin function and reduce erythrocyte life span (da Costa-Val *et al*., 2007).

In the current study, most dogs (n= 16; 84.2%) with leishmaniasis had no changes in the total leukocytes count. A great variability in the leukogram of dogs with Leishmania infection was observed in this study and other studies (Anosa and Idowu, 1983; Moreno *et al*., 1998; Koutinas *et al*., 1999; Juttner *et al*., 2001 andAmusategui *et al*., 2003). Some animals displayed an excess of certain types of leukocytes while a few others presented a deficit of the same cells.The most frequently reported alterations of the leukogram include leukopenia and an inversion of the leukocytes count, due to an increase of lymphocytes and a drop in the number of neutrophils (Amusategui *et al*., 2003).In our study, lymphocytosis was the most frequently alteration found in the leukogram of leishmania infected dogs (21%) and the number of lymphocytes was significantly higher in the infected dogs than thosewhich were not infected (p<0.05). persistent antigenic stimulation of chronic infections like leishmaniasis may cause lymphocytosis in dogs (Schultze *et al*., 2000).

In the present study, 26.3% (5/19) of infected dogs showed hyperproteinemia. Blood protein alterations are one of the most frequently reported alterations of biochemical panel in Leishmania-infected dogs (Slappendel, 1988; Palacio *et al*., 1995; Lester and Kenyon, 1996; Ciaramella *et al*., 1997 and Koutinas *et al*., 1999). It seems that blood protein alterations are linked to a polyclonal humoral immune response, which leads to raised protein levels in plasma. This can be observed by an increase of the total plasma proteins with hyperglobulinemia, hypoalbuminemia and altered Albumin: Globulin ratio (Reis *et al*., 2006).

Dogs with leishmaniasis often show clinical signs of bleeding such as epistaxis, haematuria and haemorrhagic diarrhea. It is suggested that bleeding is caused by the presence of ulcerations, primary or secondary haemostasis alteration and haemorrhagic diathesis followed to hyperglobulinaemia,paraproteinaemia, and azotaemia (Di Loria *et al*., 2006). In contrast to Di Loria *et al*. (2006) and Ciaramella *et al*. (1997 and 2005) who found 27.3-29.3% thrombocytopenia in Leishmania-infected dogs, in our study, thrombocytopenia was observed in just 1 dog (5.3%). Abnormal platelet functions (thrombocytopathy) such as deficiency in platelet aggregation have also been found in many infected dogs (Ciaramella *et al*., 1997 and 2005). Thrombocytopenia and thrombocytopathy may result from abnormal vascular wall due to vasculitis, altered thrombopoiesis, and increase in platelet destruction and/or following to renal or hepatic failure (Di Loria *et al*., 2006).

In conclusion, there is a relatively high sero-prevalence rate (8.6%) for *L. infantum* among dogs in Mashhad, Iran. A high proportion of sero-positivity for leishmaniasis was among owneddogs. This indicates that parallel to the stray dogs; owned dogs can also play an important role in the epidemiology of this zoonotic disease. In addition, determination of Leishmania infection sero-prevalence in the domestic dog population could be a helpful way to follow the progress of the disease in endemic areas.The most frequent hematological finding in the studied Leishmania- infected dogs was non-

regenerative anemia, which could be taken into account when dealing with diagnosis of canine leishmaniasis in endemic regions.

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شیوع و فاکتورهای خطر لیشمانیوز سگ‌ها در شهرستان مشهد، شمال شرق ایران

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چکیده

مطالعه حاضر جهت تعیین شیوع سرمی و فاکتورهای خطر لیشمانیوز در یک جمعیت از سگ‌های خانگی و ولگرد مشهد (شمال شرق ایران) انجام گردید. از مجموع ۳۰۰ نمونه سرمی جمع آوری شده از سگ‌ها، ۸/۶۷ درصد (۲۶/۳۰۰) آن‌ها در روش ایمنوفلورسانس غیرمستقیم (IFAT) آلودگی با لیشمانیا اینفانتوم را نشان دادند. میزان شیوع سرمی در سگ‌های خانگی و ولگرد به ترتیب ۹/۵ (۱۹/۲۰۰) و ۷ درصد (۷/۱۰۰) بود. تفاوت معناداری بین گروه‌های سنی مختلف مشاهده گردید (P<0.05) و سگ‌های کمتر از یک سال سن بیشترین شیوع سرمی را نشان دادند (۱۷/۴ درصد). شیوع سرمی لیشمانیا در دو جنس نر و ماده به ترتیب ۱۰/۴۳ درصد (۱۷/۱۶۳) و ۶/۵۷ درصد (۹/۱۳۷) و در سگ‌های نژاد کوچک و بزرگ به ترتیب ۱۰/۵ درصد (۶/۵۷) و ۸/۲ درصد (۲۹/۲۴۳) تعیین گردید. تفاوت معنی داری از لحاظ جنس و نژاد در سگ‌های مورد مطالعه مشاهده نشد. تعداد لنفوسیت‌ها در سگ‌های آلوده با لیشمانیا بیشتر از سگ‌های غیر آلوده بود (P<0.05). مقادیر PCV کم خونی غیر جبرانی را در تعداد زیادی از سگ‌های آلوده با لیشمانیا نشان داد (۵۲/۶ دردصد). با توجه به نتایج بدست آمده از مطالعه حاضر می‌توان نتیجه گیری نمود که عفونت لیشمانیوز از شیوع سرمی نسبتاً بالایی در سگ‌های خانگی و ولگرد شهرستان مشهد برخوردار است. به علاوه کم خونی غیر جبرانی فراوان ترین یافته خون شناسی در سگ‌های آلوده با لیشمانیا است، که بایستی در هنگام تشخیص لیشمانیوز سگ‌ها در مناطق بومی مد نظر قرار گیرد.

واژگان کلیدی: لیشمانیوز سگ‌ها، مشهد، ایران، خون شناسی